

AMENDMENTS TO THE SPECIFICATION

Please replace the paragraph at page 38, lines 12-22, with the following paragraphs:

In addition, the human total RNA hybridization showed that the signal intensities are comparable for all three capture concentrations (Fig. 6b). Capture oligos were spotted on CodeLink slides. 1 ug of human total reference RNA was hybridized on a microarray in a mixture containing 30%-50% formamide, 4x SSC, 0.04% Tween, 0.02% SDS, at 40°C for 2h. After hybridization, the arrays were washed in 0.5 M NaNO₃/0.02% Tween/0.01%SDS (3X) at RT, 0.2XSSC, 10seconds (2X), and spin dry. The arrays were further hybridized with 1nM dT 20mer-gold nanoparticle probe in a mixture containing 20%-40% formamide, 4x SSC, 0.04% Tween, 0.02% SDS, at 40°C for 30 min. The arrays were washed in 0.5 M NaNO₃/0.02% Tween/0.01%SDS (3X) at RT, 0.5 M NaNO₃ (2X). The slides were subjected to silver stain (5.5 min) to obtain scatter signal. Fig. 6c is the array layout for arrays shown in Figure 6 and Figure 7 (below).

Please replace the paragraph at page 38, line 23 to page 39, line 8, with the following paragraph:

The effect of different detergent combinations on hybridization was then tested. Human test arrays were hybridized with 0.1ug of total human universal reference RNA (BD Bioscience Clontech) with fixed Tween 20 concentration (0.04% in hybridization mixture) and titrated SDS concentrations from 0.001% to 0.1%. The hybridization signal was higher as the SDS concentration increased from 0.001% to 0.02% (Fig. 7). The experimental conditions were as follows: Capture oligos (at 1uM, 3uM and 9uM) were spotted on CodeLink slides. 0.1 ug of human total reference RNA was hybridized on a microarray in a 5ul of mixture containing 50% formamide, 4x SSC, 0.04% Tween, and different SDS concentrations as indicated, at 40°C for 1.5h. After hybridization, the arrays were washed in 0.5 M NaNO₃/0.02% Tween/0.001%SDS (3X) at RT, 0.2XSSC, 10seconds (2X), and spin dry. The arrays were further hybridized with 1nM of dT 20mer-gold nanoparticle probe in a mixture containing 30% formamide, 4x SSC, 0.04%

Tween, and different SDS concentrations as indicated, at 40°C for 30 min. The arrays were washed in 0.5 M NaNO₃/0.02% Tween/0.001%SDS (3X) at RT, 0.5 M NaNO₃ (2X). The slides were subjected to silver stain (5.5 min) to obtain scatter signal. The increased hybridization signal may be due to the reduced non-specific RNA target binding to the slide surface which results in improved hybridization kinetics. Fig. 7b is a plot of signal intensity of beta actin spots printed at 1uM. For Fig. 7a, the net hybridization signal of beta actin spots (capture oligo at 1uM) was measured and plotted for each SDS concentration.

Please replace the paragraph at page 40, lines 8-15, with the following paragraphs:

To examine the sensitivity of RNA detection and establish a correlation between signal intensity and mRNA copy numbers, a titration of bacterial control RNA was performed with improved assay conditions. The control RNA spike 4 (1,000 bases, in vitro transcript, Ambion cat# 1780 ArrayControl™ RNA Spikes) was included in human total RNA mix with dilutions from 0.5 pg/hybridization to 0.5fg/hybridization. The hybridization results showed lower limitation of detection at 5-50fg of mRNA (equivalent to 10,000-100,000 copies) with overnight hybridization (Fig. 9(a)) and 50-500fg (equivalent to 100,000-1000,000 copies) with 2 hour hybridization (Fig 9b).

The net signal intensity and signal/background ratio of control gene #4 were plotted. Fig. 9a is RNA hybridization for 24 hours. Fig. 9b is RNA hybridization for 2 hours. Experiment conditions: Capture oligos (control gene number 4, from Ambion) were spotted on CodeLink slides. Control RNA number 4 (in vitro transcript) titration (experiment was performed to evaluate the sensitivity of RNA detection. For 24h hybridization, the input of RNA molecules ranged from 1,000,000 per hybridization to 1,000 per hybridization. For 2h hybridization, the input of RNA molecules ranged from 10,000,000 per hybridization to 10,000 per hybridization. The RNA was hybridized on microarrays in a 5ul of mixture containing 48% formamide, 4XSSC, 0.04% TWEEN, and 0.01% SDS, at 40°C for 2h or 24h as indicated. After hybridization, the arrays were washed in 0.5 M NaNO₃/0.02% TWEEN/0.01% SDS (3X) at RT, 0.2XSSC, 10 seconds (2X), and spin dry. The arrays were further hybridized with 1nM of dT 20 mer-gold nanoparticle probe in a mixture containing 35% formamide, 4XSSC, 0.04% TWEEN,

and 0.01% SDS, at 40°C for 30 min. The arrays were washed in 0.5M NaNO₃/0.02% TWEEEN/0.01% SDS (3X) at RT, 0.5 M NaNO₃ (2X). The slides were subjected to silver stain (5.5 min) to obtain a scatter signal.